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Sediment Core Sampling

Water Analyses: (Take precautions to minimize aeration for all samples.)

Stable Isotope Probing

- Remove water over sediment surface using a 25 mL pipette.
- Transfer water into a 60 mL syringe attached to a 0.22 µM Sterivex filter with a needle attached to the other end.
- Fill Evacuated 125 mL bottles.
- Collect samples from two of the twelve sediment cores retrieved from each location.
- Store bottles at 4°C.

Nutrient and VOA analyses

VOA:

- Collect 40 mL overlying water using a 25 mL pipette.
- Transfer water into a 40 mLVOA vial containing 50 µL of full-strength HCl.
- Collect two samples, one from each of two separate sediment cores.
- Store bottles at 4°C.

Nutrient:

- Collect 100 mL overlying water using a 25 mL pipette.
- Transfer water into a 125 mL HDPE (plastic) bottle.
- Collect two samples, one from each of two separate sediment cores.
- Store bottles at -80°C.

Culturing

- Collect 10 – 20 mL water overlying sediment cores using a 25 mL pipette.
- Transfer water into 40 mL VOA.
- Store samples at 4°C.

AODC

- Collect 18 mL water overlying sediment cores using a 25 mL pipette.
- Transfer water into a 50 mL Falcon tube containing 2 mL 37% formaldehyde.
- Wrap tube with parafilm.
- Store tubes at 4°C.
- *Note: Collect two samples, one from each of two separate sediment cores. If there is insufficient water for two samples of 18 mL each, then collect less water (either 9 mL or 13.5 mL) and transfer into tubes containing enough formaldehyde to yield 10% final volume (1 mL or 1.5 mL if using 9 mL or 13.5 mL water, respectively).

PLFA

- The Sterivex filter used for SIP water processing will continue to be used to filter remaining volumes of water for PLFA. See the table below for estimated total volumes of water to process for PLFA analysis.
- Store filters at -80°C.

Nucleic Acids (RNA/DNA)

- Filter water onto MoBio filters in volumes indicated in the table below.
- Process water from different sediment cores onto separate filters.
- Fold filters in half using sterile forceps, pick up folded filter with a second pair of forceps and twist gently to insert folded filter into a 15-mL tube.
- [Transfer filters into RNALater solution (10 mL in 15-mL Falcon tubes).]
- Store filters in tubes at -80°C.

- Note: When sampling larger volumes of water (e.g., greater than 200 mL), it may make more sense to find an alternate way to move the water from above the core surface to the filter units. Keep in mind the desire to minimize disturbance of the layer of water above the sediment and the surface layer of sediment.

Single cell genomics

- Collect three 1 ml samples and preserve with 15% cold glycerol (pre-aliquoted into tubes).
- Store samples at -80°C.

Sediment core samples

- Will be processed at LBNL.
- Store 2 cores without overlying water upright at -80°C until they are frozen solid. (Process water as noted above.) For shipping, these cores can be laid on their sides.

- Store 2 cores with overlying water (if possible) upright at 4°C. Ship upright if possible. If water does not stay in cores using sealed caps, remove the water and store in glass amber jars, filling the jars completely if possible. If there is very little water, smaller glass jars may be used. Ship cores upright if possible.

Sampling volumes given various amounts of water above one sediment core.

If 475 mL	mL
SIP	125
VOA	40
Nutrient	100
Culture	10
AODC	18
PLFA (includes 125 from SIP)	200
RNA/DNA	100
Single-cell genomics	3
Total:	471

If 950 mL	mL
SIP	125
VOA	40
Nutrient	100
Culture	10
AODC	18
PLFA (includes 125 from SIP)	380
RNA/DNA	400
Single-cell genomics	3
Total:	951

If 1425 mL	mL
SIP	125
VOA	40
Nutrient	100
Culture	10
AODC	18
PLFA (includes 125 from SIP)	600
RNA/DNA	650
Single-cell genomics	3
Total:	1421

Referenced Info:

http://www.deep-sea-frontier.eu/front_content.php?idart=621

"In the water column 10^4 cells per cm3 are the typical densities of microbial cells in the water column. Volumetric abundances abruptly rise to 10^9 cells cm-3 within the surface sediments. Even in the most energy poor, ultra-oligotrophic region of the South Pacific Gyre, cell counts exceed 10^6 cells cm-3 at surface sediments. Below the surface meter of sediment, volumetric cell counts decline in a logarithmic fashion, reaching values of 10^4 cells cm-3 only after tens to hundreds of meters of sediment depth. Correspondingly, microbial activities, excluding hotspots or deeply buried high-energy interfaces, decline even more precipitously with increasing depth. Thus, the surface and near-surface seafloor represents a plate of high microbial abundance, high microbial diversity, high microbial activity separating the vast deep biosphere habitat from the deep ocean water masses that dictate climate." **Tim Ferdelman, MPI, Germany** ***Judith McKenzie, ETH Zürich, Switzerland***